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Non-cytotoxic conductive carboxymethyl-chitosan/aniline pentamer hydrogels

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ABSTRACT

Cytocompatible electrically conducting hydrogels based on amphoteric carboxymethyl chitosan (CMCS) and aniline oligomers with bioactive molecule delivery properties were presented. A series of conductive CMCS hydrogels with different aniline pentamer (AP) content were synthesized by a one-pot reaction with the combination of grafting and crosslinking reaction via glutaraldehyde. The conductivities of the swollen hydrogels are between 1.14×10^{-4} and 4.23×10^{-4} S/cm by tuning the AP content. Swelling ratio was controlled by the AP content and crosslinking degree of the hydrogels. The hydrogels showed absorption capacity of diclofenac sodium (DCS) as a model molecule and released DCS in a controlled manner. Morphologies and mechanical properties of the hydrogels were characterized by SEM and rheometer, respectively. The biocompatibility of the hydrogels was confirmed by C2C12 myoblast cells using Live/Dead assay and Alamar blue assay. These conducting hydrogels with controlled release capacity as bioactive scaffolds have potential application for tissue regeneration.

1. Introduction

Tissue engineering aims to construct live functional substitutes to repair damaged or dysfunctional tissues by using a combination of cells and polymer scaffolds. Three dimensional (3D) porous scaffolds provide a temporary support for cell growth and promote neo-tissue regeneration process [1–3]. The polymer 3D scaffolds mimic many roles of extracellular matrices (ECM) which are consisted of various amino acids and sugar-based macromolecules [4,5]. Various types of scaffolds have been developed for tissue engineering applications. Hydrogel is one of the materials widely used in tissue engineering due to their rubbery nature similar to the soft tissues, their control of diffusion of oxygen, nutrients and other bioactive molecules and their excellent biocompatibility [6,7]. Hydrogels from naturally derived polymers have potential advantages of good biocompatibility, degradability and intrinsic cellular interaction with tissue [8,9]. As one of the most abundant biopolymers chitosan has been widely used in biomedical fields because of its unique polycationic nature [10–14]. However, the poor solubility of chitosan in neutral water limits its application. Carboxymethyl chitosan (CM-CS), a natural amphoteric polyelectrolyte derived from chitosan, is not only soluble in neutral and basic water, but also has unique chemical, physical and biological properties. It has found wide applications in wound healing, tissue engineering, drug delivery system and gene therapy [15–18]. It contains large amount of —COOH and —NH_2 groups which can be further functionalized.

The 3D scaffolds should regulate the cell adhesion as well as migration and differentiation in sophisticated tissue engineering. Previous studies demonstrated that electrical stimulations can regulate cellular activities, including cell attachment, migration, proliferation and differentiation [19,20]. This indicates that conducting polymers have great potentials in tissue engineering applications, since the regulation of cellular activities is critical for the regeneration of damaged tissue. Conducting polymer composites and hydrogels based on polyaniline [21–23], polypyrrole [24,25], polythiophene and their derivatives [26–28] have been developed [29–31]. However, the poor solubility and non-degradability of conducting polymers greatly restricted their applications in tissue engineering. In contrast, oligomers of aniline have well-defined

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structure, good solubility and electroactivity as well as good biocompatibility [32–36]. Moreover, their amine-capped structure makes them possible to be copolymerized or grafted with other monomers or polymers to prepare functional materials as shown in our previous work [37–39].

The localized and temporally controlled delivery of bioactive molecules such as drugs, protein and growth factors is very important to improve the clinical efficacy. In advanced tissue engineering processes, the biodegradable scaffolds should serve as both a three-dimensional (3-D) substrate and a bioactive molecules delivery depot to enhance cellular activity [40–42]. The development of temporally and spatially controlled delivery systems that integrates the biodegradable scaffolds is still a clinical challenge. The goal of this work is to design and synthesize a series of amphoteric conducting hydrogels with bioactive molecule delivery properties based on CMCS and aniline pentamer for tissue engineering applications. We used diclofenac sodium (DCS, a nonsteroidal anti-inflammatory compound with a UV–Vis absorption at 276 nm) as a model molecule to study the release property of the conducting hydrogels. The hydrogel preparation, swelling behavior, conductivity, surface morphology, mechanical properties and release properties of the hydrogels were investigated. C2C12 myoblast cell adhesion and proliferation for the hydrogel were also evaluated.

2. Materials and methods

2.1. Materials

Chitosan (CS, weight molecular weight 100,000–300,000 Da) was obtained from J&K Scientific Ltd. Glutaraldehyde (GA, Aldrich) was diluted to 5 wt% aqueous solution before use. Monochloroacetic acid, N-phenyl-1, 4-phenylenediamine, phenylamine, ammonium persulphate ($(NH_4)_2S_2O_8$), ammonium hydroxide (NH_4OH), sodium hydroxide (NaOH), isopropanol, anhydrous ethanol, tetrahydrofuran (THF), diclofenac sodium (DCS) and dimethyl sulfoxide (DMSO) were all purchased from Aldrich and were used as received. Carboxymethyl chitosan (CMCS) was prepared as described previously [43]. The substitution degree of CM-chitosan was 89% by potentiometric titration [44].

2.2. Synthesis of aniline pentamer

Aniline pentamer was synthesized according to Ref. [45], and synthesis route is shown in Fig. 1 in Supporting Information (SI). Briefly, 0.2 mol aniline and 0.1 mol p-phenylenediamine was oxidized by 0.2 mol $(NH_4)_2S_2O_8$ in 1 mol/L HCl and acetone mixture to produce amino-capped aniline trimer (Fig. 1 in SI). Amino-capped aniline trimer: ^{1}H NMR (400 MHz, DMSO-d_6) δ = 6.96 (s, 4H, Ar–H), δ = 6.81 (d, 4H, Ar–H), δ = 6.62 (d, 4H, Ar–H), δ = 5.43 (s, 4H, –NH_2). This result agrees well with the Ref. [46].

For the synthesis of aniline pentamer, 2.28 g amino-capped aniline trimer and 3.04 g N, N-diphenylamine were dissolved in 80 mL DMF. 40 mL water and 20 mL 36% HCl were then added to the above solution with vigorous stirring. After reaction at room temperature for 4 h, the HCl-doped aniline pentamer was obtained by filtration, and then washed by a mixture of DMF/H_2O. The product was dedoped in a 100 mL 1 mol/L NH_4OH for 30 min to produce aniline pentamer in emeraldine state. The emeraldine aniline pentamer was reduced by phenylhydrazine, and was precipitated in a H_2O/ethanol mixture. The leucoemeraldine aniline pentamer was collected by filtration and washed thoroughly with H_2O/ethanol mixture. The product was finally dried in a vacuum oven. The ^{1}H NMR spectrum was shown in Fig. 2 in SI. Leucoemeraldine aniline pentamer: ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.73 (s, 1H, Ar–NH), δ = 7.52(s, 1H, Ar–NH), δ = 7.37 (s, 1H, Ar–NH), δ = 7.14 (s, 1H, Ar–NH), δ = 7.13–7.11 (m, 2H, Ar–H), δ = 6.98–6.95 (m, 2H, Ar–H), δ = 6.90–6.83 (m, 10H, Ar–H), δ = 6.77–6.75 (m, 4H, Ar–H), δ = 6.67–6.65 (m, 1H, Ar–H), δ = 6.51–6.49 (m, 2H, Ar–H), δ = 4.63 (s, 2H, Ar–NH). ^{13}C NMR (100 MHz, DMSO-d_6), δ = 145.61 (Ar–C), 142.53 (Ar–C), 139.47 (Ar–C), 139.39 (Ar–C), 138.56 (Ar–C), 135.86 (Ar–C), 135.71 (Ar–C), 134.29 (Ar–C), 133.67 (Ar–C), 129.04 (Ar–C), 120.09 (Ar–C), 120.27 (Ar–C), 119.16 (Ar–C), 118.99 (Ar–C), 117.72 (Ar–C), 117.04 (Ar–C), 116.67 (Ar–C), 116.23 (Ar–C), 114.88 (Ar–C), 114.38 (Ar–C). This data agree well with the previous results [47].

2.3. Synthesis of CMCS-AP hydrogels

The conductive hydrogels were synthesized in a one-pot reaction as shown in Fig. 1. 0.05 g CMCS was dissolved in 2 mL of distilled water. Different amounts of AP (see Table 1) which dissolved in 1 mL DMSO and appropriate amounts of GA (Table 1) were added into the above solution and stirred vigorously. The reaction was then kept for 12 h at room temperature without stirring. The hydrogel was immersed in H_2O/THF mixture (Vol:Vol = 90:10) for 2 days to extract the unreacted AP. The H_2O/THF mixture was changed every 6 h. All the extracted solution was collected and was used for UV–Vis test. After that, the hydrogels were soaked into distilled water to exchange with the THF in the hydrogels with changing the water for several times. The hydrogels were then dried in air at room temperature and stored in a desiccator.

Hydrogels with same crosslinking degree but containing different weight ratio of AP were prepared by adding different amounts of AP into the system, and samples having 0%, 5%, 10%, 15% and 20% weight ratio of AP in the hydrogel were coded as CMCS hydrogel, CMCS-AP5 hydrogel, CMCS-AP10 hydrogel, CMCS-AP15 hydrogel and CMCS-AP20 hydrogel, as listed in Table 1.

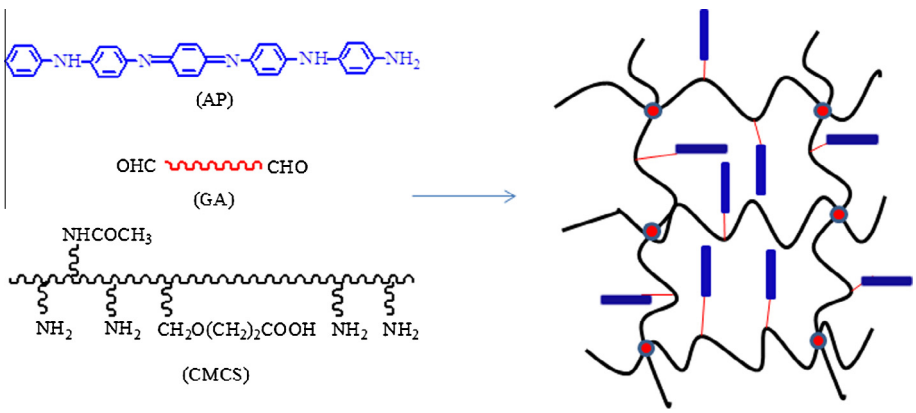

Fig. 1. Schematic synthesis of CMCS-AP hydrogels.